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A REVIEW OF THE PHYSIOLOGICAL IMPACT OF MERCURIALS

SAVANNAH RIVER ECOLOGY LABORATORY

PREPARED FOR
ENVIRONMENTAL PROTECTION AGENCY

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A REVIEW OF THE

PHYSIOLOGICAL IMPACT OF MERCURIALS

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CONTENTS

	Page
ABSTRACT	ii
ACKNOWLEDGMENTS	v
Sections	
I CONCLUSIONS	1
II INTRODUCTION	2
III ORGANISM RESPONSE TO MERCURIALS	3
SYMPTOMS	3
UPTAKE, RETENTION, EXCRETION, BIOTRANSFORMATION	6
Methyl Mercury	6
Dimethyl Mercury	12
Phenyl Mercury	14
Methoxyethyl Mercury	16
Ethyl Mercury	16
Mercuric Salts	17
Metallic Mercury	18
Comparison of Mercurials	19
IV ORGAN AND TISSUE EFFECTS	23
CENTRAL NERVOUS SYSTEM	23
KIDNEY	25
LIVER	26
HEART	27
BLOOD	27

		Page
	EYE LENS	29
	SUMMARY	29
V	CELLULAR EFFECTS	31
	INTRACELLULAR DISTRIBUTION	31
	CELL MORPHOLOGY	32
	CELL MEMBRANE	33
	CHROMOSOMES	33
	MITOCHONDRIA	34
VI	BIOCHEMICAL EFFECTS	35
	GENERAL EFFECTS	35
	CHEMICAL PROPERTIES--ORGANIC MERCURIALS VS. INORGANIC MERCURIALS	36
	INHIBITION BY MERCURIALS	36
	RATIONALE FOR THE ACTION OF MERCURIALS	39
	IN VIVO EFFECTS--RATIONALE FOR SPECIFIC ORGAN DAMAGE	39
VII	REFERENCES	43

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SECTION I

CONCLUSIONS

Studies of mercurial poisoning show that gross symptoms can be related to tissue damage. In turn, investigations of cellular changes reveal that tissue damage results from the morphological changes occurring in cells. The changes at the cellular level are more subtle in the early stages of damage, and early changes precede evidence of tissue damage. Thus, cellular changes provide a more subtle index of damage due to mercurial exposure. Relating morphological changes at the levels of the organism, tissue, and cell to biochemical effects is more difficult. Caution is stressed in trying to understand mercurial poisoning in terms of biochemical damage. The cell membrane, non-enzymatic cellular protein, and outer cell layers provide protection to the inner cells or organs. Nevertheless, researchers have theorized on probable biochemical effects which lead to the more apparent symptoms of mercurialism. Damage to Krebs' cycle enzymes and thus to the energy obtaining mechanism of the kidney, severely limits the kidney's ability to actively resorb ions. In addition, possible damage to both protein synthesis and Krebs' cycle enzymes in the brain could result in the extensive tissue damage observed in alkyl mercurial poisoning. Thus, biochemical changes can be related to other levels of organization and ultimately to the organism as the basis of the morphological and behavioral changes which characterize mercurialism.

SECTION II

INTRODUCTION

The presence of mercury has been known since prehistoric time. It was first used c. 1000 A.D. as a medication for skin diseases. Ulrich Ellenborg made the earliest survey of mercury's toxic properties in 1473.[1] The use of mercurials has extended from pharmaceuticals to agriculture and industry resulting in the broad dissemination of mercury into the environment.

Agricultural and industrial applications have given rise to the recent problem of mercury pollution. The first serious incident resulting from industrial mercury discharges occurred in Minimata, Japan, in the form of an undefined neurological disorder. The first victim was reported in 1933 but the problem eventually affected many individuals of the Minimata Bay and River areas reaching epidemic proportions in 1953.[2] A similar but smaller outbreak occurred in 1965 in Niigata, Japan.[3] The mercury problem was next discovered in Sweden, although it never caused the health menace observed in Japan. The first evidence for the detrimental effect of mercurials on the Swedish environment appeared in 1955 when a decrease in the numbers of predaceous and seed-eating birds was observed.[4] Borg et al.[4] attributed this decline to the use of seed dressings. Westermark was the first to note abnormally high levels of mercury in Swedish fish.[5] Interest in mercury in America began in March, 1970, when the Canadian Fisheries Department closed a commercial fishery on Lake St. Clair because of high mercury content in the fish.[6] Several waterways in the United States have since been closed to fishing because of high mercury levels.[7]

The detrimental impact of mercurials on natural populations and individual species has stimulated investigation of the mercury problem at other levels of biological organization. Medical descriptions of symptoms and organ histopathology are numerous.[2, 8, 9] Cellular distribution,[10] genetic effects,[11] and enzyme responses[12] to mercury have all added to the knowledge of the physiological effects of mercury. The effects at each level of organization must be related to more refined levels in order to determine the actual physiological responses to mercurials. Only when biochemical effects can be related to clinical symptoms in the whole organism will the effects of mercurials on populations and communities be fully understood.

The aim of this paper is to bring together information concerning the effects of mercurials at all levels of organization: organism, organ, tissue, cell, and molecule. It is hoped that a review of the effects of mercurials at each of these levels will clarify the total physiological impact of mercury.

SECTION III

ORGANISM RESPONSE TO MERCURIALS

SYMPTOMS

Mercury poisoning manifests itself in different ways for different routes of administration, kinds of mercury compounds, and duration of exposure. Some of the information is represented by single examples of unusual routes of entry. However, much information has been supplied by studies of industrially exposed populations and inhabitants of Minimata, Japan. These situations have supplied adequate numbers to determine predominant symptoms of poisoning by different mercurials. In addition, animal studies have supplied preliminary evidence of concentrations of mercurials necessary to cause neurological symptoms.

Mercurials can enter the body through inhalation, subcutaneous or intravenous injection, cutaneous application, or ingestion. Each route of entry can exhibit unique symptoms unobtainable from any other mode of administration. For example, subcutaneous injection of metallic mercury causes deep lesions in the area of injection.[13, 14, 15] Application of mercuric chloride or metallic mercury to the skin may cause dermatitis, discoloration of the fingernails, systemic poisoning, purpura, petechiae[15] and skin lesions.[16] Corrosion of the conjunctiva and cornea may result from eye contact. Ingestion of mercuric chloride causes a burning metallic taste, thirst, and soreness of the pharynx.[15] One-half gram of mercuric chloride can be a lethal dose causing shock, ulceration of the gastro-intestinal tract, and destruction of the tubular epithelium of the kidney.[17] Mercury fulminate applied to the skin may cause dermatitis and, to the eye, conjunctivitis and edema of the lid.[15] Mercury oxycyanide when locally applied gives rise to corrosion of the membranes and when ingested, abdominal pain, vomiting, and diarrhea. Phenylmercuric acetate applied to the skin causes effects similar to burning.[18] Vapour exposure may cause mercurialentis.[19] Organic mercurials affect the central nervous system while inorganic forms primarily affect kidney function. Methyl mercury, mercurial diuretics, and phenylmercuric acetate will be considered under organic mercurials. Inorganic mercurials reviewed will include mercuric chloride, metallic mercury, mercury fulminate, and mercury oxycyanide.

The most frequent route of administration of methyl mercury is ingestion of protein bound methyl mercury in food items. The most extensive discussion of the clinical effects of methyl mercury resulted from data on victims of "Minimata disease". The earliest symptoms include fatigue, headache, and reduction in powers of concentration and memory.[18] A latent period of two to seven months may be required

before nervous disorders become apparent.[18] The early neurological symptoms in acute or subacute cases include numbness of the extremities and lips, slurred speech, unsteady gait, deafness, concentric constriction of the visual field, tremor, and slight mental disturbances.[2, 8, 9] These symptoms occurred in 70-100% of the cases reported in Minimata.[2] Muscle involvement often leads to spasticity and rigidity with muscle stretch reflexes exaggerated or depressed.[8, 9] Hypethesia and convulsions are sometimes present.[8, 9] Tremors include choreoathetosis, myoclonus, and coarse resting and action tremors.[8] Mental disturbances range from increased emotional lability to the mental confusion of the seriously ill. The former is characterized by alternating euphoria and depression and the latter by periods of drowsiness and stupor interchanging with periods of shouting and restlessness.[9] Children under ten years appear to be more susceptible, and exposed fetuses may develop cerebral palsy symptoms at birth.[2] In severely affected patients, generalized muscle wasting may occur with the patient becoming terminally comatose. Death usually results from intercurrent infection, pneumonia, or inanition.

Symptomatic behavioral changes have been observed in animals exposed to methyl mercury. Behavioral changes in fish include increased difficulty in maintaining their balance, irregular respiration,[20] and impairment of learning.[21] Mice exhibit a characteristic hind-limb crossing behavior after exposure to methyl mercury[22] and, at less critical levels, show changes in swimming behavior.[23] Chicks also show impaired learning when exposed to low levels of mercury as embryos.[24] More acute dosage levels cause impaired muscular coordination.[25]

Little is known of methyl mercury levels required to cause neurological symptoms. Berglund and Berlin[26] have reported that the body burden at which a toxic level is reached in the brain varies with species. However, they have hypothesized that a level of eight micrograms per gram of brain is required to elicit neurological signs in man. Suzuki[27] has indicated that a peak concentration of twenty micrograms per gram wet brain tissue is the threshold of neurological symptoms. At a peak of thirty micrograms per gram of wet brain tissue, death follows neurological signs. Skerfving[28] and Hammond[29] gave a 0.2 μg/g whole blood as a minimal blood concentration for neurotoxic exposure. This is equivalent to ingesting 4 μg/Kg weight daily.

Tokuomi[2] divided methyl mercury poisoning into four groups based on its course and prognosis. The first is the common form with the more prevalent symptoms indicated earlier (numbness of extremities, slurred speech, unsteady gait, deafness, constriction of visual field, tremor, mental disturbances). Although some cases later develop characteristics of other forms, the course of the common type is slow improvement with some sequelae. In the second type,

acute fulminate, the patient lapses into unconsciousness and shows involuntary movements marked by agitation and shouting. Death due to pneumonia and high fever usually follows shortly. In the third form, chronic with irritation, the symptoms are similar to the common form but in later stages the patient shows psychic excitement and pyramidal symptoms. Some show frequent convulsions. Individuals with the fourth form, chronic with contractions, show common symptoms, but the joints become fixed by contractures in abnormal positions. All individuals Tokuomi reviewed showing the last two forms died.

Organic mercurial diuretics are administered intravenously and sensitization to them may give rise to toxic or allergenic reactions. Immediate effects may include pallor, stertorous respiration, jerky movements and collapse. Although no immediate effects are apparent, one or two hours after injection the patient may develop chills, fever, dypsnea, asthmatic attacks, pulmonary edema, and prostration. This condition may lead to death. After several tolerated injections, an additional one may yield giddiness, transitory dypsnea, orthopnea, apprehension, substernal pain, brachycardia, syncope, fall in blood pressure, collapse, and cyanosis. The patient may be drowsy, mentally confused, or even delirous. The allergenic reaction may include chills, fever, cutaneous eruptions, and exfoliative dermatitis. Mercurial diuretics may cause tubular nephrosis.

The most common clinical characteristics of poisoning by inorganic mercury salts are tremor and erethism.[16] Tremor is first present only during excitement. It is first observed in the hands, then the eyelids, tongue, arms, cheeks, head, and eventually the legs are involved. A blue line on the gums may appear, and salivation sometimes increases. Stomatitis and gingivitis with ulceration of the mucous membranes occur but are not common.

Von Oettingen[15] indicated that ingestion of mercuric chloride is not usually followed by evidence of nervous system involvement as in alkyl mercurials. He stated that prolonged use could lead to chronic poisoning with sudden fever, glandular swelling, scarlatiniform eruptions, edema, anuria, and possible nephrosis. Kanzantis et al.[30] indicated that albuminuria and the nephrotic syndrome may result from prolonged mercury exposure. The nephrotic syndrome manifests itself with heavy albuminuria, hypoalbuminaemia, and hypercholesterolemia. The albuminuria may be due in part to protein excretion with cytoplasmic blebs from the proximal tubule instead of glomerular malfunction.[31]

Von Oettingen[15] discussed the effects of metallic mercury including two paths of entry, the skin and the gastro-intestinal tract. The skin may show inflammatory reactions such as eczema, petechial hemorrhages, and excessive perspiration. The fingernails could show desquammation and dystrophy. Subcutaneous globules of mercury may cause granulomas. Disturbances to the gastro-intestinal tract result

in hunger or lack of appetite, the latter resulting in weight loss. Increased salivation and a metallic taste in the mouth may be noted. The gingiva may be spongy and ulcerated. The teeth may erode and fall out. Stomatitis may develop to an ulcerative or gangrenous state. Rhinitis, anosmia, and necrosis of the maxilla can occur rarely. The nervous system is eventually involved giving rise to the neuromotor and nervous disturbances previously described. Renal involvement can occur with nephritis, polyuria leading to anuria, and uremia.

Mercury fulminate, when applied to the skin, may yield a form of dermatitis which is difficult to heal. When applied to the eyelids, it causes conjunctivitis. Inhalation or ingestion can cause chronic mercury poisoning.

Mercury oxycyanide applied locally can result in the corrosion of membranes and, after absorption, systemic effects. Ingestion causes abdominal pain, vomiting, diarrhea and eventually death. Prolonged cases may involve kidney damage.[15]

In summary, prolonged exposure to all forms of mercury can lead to mercury poisoning. The two major responses to mercury poisoning involve neurological and renal disturbances. The former is characteristic of organic mercurial poisoning where liver and renal damage are of relatively little significance.[32] The latter is characteristic of inorganic mercurial poisoning.[32]

UPTAKE, RETENTION, EXCRETION, BIOTRANSFORMATION

Many investigators have studied the uptake, retention, distribution, and excretion of various mercurials. Methyl, dimethyl, phenyl, and methoxyethyl mercurials are most frequently studied organic forms. Several researchers have also included ethyl mercurials in their work. Inorganic salts (mercuric nitrate, mercuric chloride, mercuric hydroxide) and mercury vapor are the most frequently studied inorganic mercurials. Data on various routes of entry and dosage levels are included in the literature. In addition, several researchers have investigated biotransformation of organic mercurials, the *in vivo* formation of mercuric ions from metallic mercury, and *in vivo* methylation of inorganic mercurials.

Methyl Mercury

Studies of the distribution and excretion of methyl mercury have considered single and multiple exposure from several routes of entry (oral, intravenous and subcutaneous injections). Mammals (pigs, mice, rats), poultry, and aquatic vertebrates (eels, flounders, pike) have been studied.

Methyl mercurials are typically distributed rather evenly among organs in rats.[33] Berlin and Ullberg[34] traced the distribution after single intravenous injections of methyl mercuric dicyandiamide (0.5 mg/kg) in mice. They found that soon after injection (up to one hour) the greatest concentration of mercury was in the blood. They noticed early accumulation in the kidney (especially in the cortex), the liver, pancreas, mucosa of the alimentary canal, the gall bladder, and the urinary bladder. After four hours, the concentration in the blood was still high. Mercury was first found in the muscles after four hours. The liver accumulation had also reached its maximum at this time, and mercury was found in the intestinal contents. By the end of the first day after injection, mercury was rather uniformly distributed. However, none was found in the bony skeleton and little in the central nervous system. Some accumulation was seen in the lacrimal and salivary glands. The kidney and liver concentrations were still well above the average body concentration. On the fourth, eighth, and sixteenth days, Berlin and Ullberg[34] reported a decline of mercury concentration by a factor of two to four in all organs except the brain and spinal cord where the mercury content more than doubled. After sixteen days, the brain concentration was exceeded only by that of the colonic mucosa and renal cortex. Swensson et al.[35] also found that methyl mercury was fairly uniformly distributed. They found mercury in the kidney after 32 days. Swensson and Ulfvarson[36] stated that the distribution in poultry is the same as in rats.

Some investigators have considered transfer to the fetus and fetal distribution. Berlin and Ullberg[34] found earliest traces in the fetus of mice after four hours. By the end of the first day after injection of the mother, the fetal concentration was equal to that of the mother. The distribution within the fetus was the same as in the mother except that more mercury was seen in the fetal skin. Suzuki et al.[37] reported even distribution between the head and body of the fetus. They also found a similar concentration in the placenta. However, less mercury was found in the amniotic membrane. They found that the quantity of mercury transferred to a mouse litter was proportional to the number of fetuses in the litter.

The main routes of excretion of intravenously injected methyl mercury are the feces and urine. Ostlund[38] showed that negligible amounts of mercury were exhaled by mice while 40% was excreted in the feces and 14% in the urine for the first seven days after injection. Swensson and Ulfvarson[36] found that only 20% of the injected dose was excreted by all routes in the first ten days after injection in poultry. Swensson and Ulfvarson[36] approximated the excretion rate in poultry to be 3.7×10^{-6} day^{-1} (μg Hg/kg wt).

Swensson and Ulfvarson[36] and Berglund and Berlin[26] stated that the excretion rate of methyl mercury fitted an exponential relationship if extremely toxic values were not included. Ostlund[38] found a

similar relationship in mice. He considered the mercury accumulated in the fur and skin as mercury excreted. Swensson et al.[35] found that excretion of methyl mercury began almost immediately after injection and then decreased nearly parallel with blood concentration.

Since ingested methyl mercury must enter the body by absorption through the gastro-intestinal tract, uptake rates have been investigated. Berglund and Berlin[26] stated that greater than 90% of the ingested methyl mercury is absorbed in the digestive tract for rats and man. Aberg et al.[39] indicated that the main uptake of absorbed mercury in the organs of the abdominal cavity. Accumulation follows an exponential curve.[26,39]

Distribution of single doses of orally administered methyl mercury compounds have been described in several organisms. Aberg et al.[39] described distribution in man. They found mercury in the blood fifteen minutes after administration and concentration in the blood corpuscles was already ten times that of the plasma. The maximum blood concentration was reached three to six hours after administration. The corpuscle to plasma ratio remained the same for 24 days. The main uptake was in the abdominal cavity with localization in the liver. Some uptake was observed in the head with possible localization in the cerebellum. Glomski and Brody[40] demonstrated the cerebellar localization more clearly. The pons usually attained the second highest mercury concentration in their study while the white matter was lowest in mercury concentration. After 40-50 days, only 0.12% of the dose/gram was found in the hair. No mercury was ever detected in the sperm.

Piper et al.[41] studied the distribution of an oral dose in pigs. They found diffuse distribution with a small dose. The largest concentration was generally found in the renal cortex (19.3%) and the second highest in the liver (13.6%). The next highest concentration varied among the renal medulla (6.8%), psoas muscle (6.0%), and the cerebrum (5.9%). Klein et al.[22] and Newberne et al.[42] also observed the highest concentration in the kidney after ingestion of methyl mercury by rats while Fimreite and Karstad[25] observed the highest level in the liver of hawks. The liver and kidneys are sites of highest concentration in duck and seal.[43] Even when small doses were given, mercury was observed on day 32.[41] Piper et al.[41] found that the distribution of methyl mercury was stabilized by day 7 after administration and remained so until day 35, the last day they sampled. The cerebrum had the largest concentration of the central nervous system, and the spinal cord had the smallest. The distribution in the cerebellum, brainstem, and spinal cord was fairly constant from days 7 to 35. The cerebrum preferentially retained mercury until day 13.

The distributions of mercury in plants and invertebrates have been studied little. Vernberg and O'Hara[44] have provided some information

on the fiddler crab. Gill tissue accumulated more than the hepatopancreas and most uptake in the gill tissue occurred during the first 24 hours of exposure. The hepatopancreas concentration rose throughout the period studied. Uptake was the same under a variety of salinity and temperature combinations.

Several algae and molluscs living in water receiving an industrial effluent have been investigated.[43] Thallose algae (Porphyra, Ulva) took up more mercury than other types. The highest mercury concentration in Fucus was found in the stipe and holdfast. Mercury concentration in the thallus gradually increased toward the apex. Jones et al.[43] also considered several molluscs (Littorina, a herbivore; Mytilus, a suspension feeder; and Nucella, a carnivore). Appreciable mercury was present at all trophic levels. In Mytilus, the ctenida contained the greatest concentration and appeared to be the area of uptake and release.

The main route of excretion of ingested methyl mercury is the feces, the same route found for injected methyl mercury. Aberg et al.[39] found that in man 13-14% of the ingested dose was excreted in the first ten days after administration. The urine contained only 0.18-0.27% in a comparable period. After 49 days, 33-35% of the dose had been excreted in the feces while only 3.3% had passed in the urine. Aberg et al.[39] stated fecal and urinary excretion were the only two significant routes. In the flounder, pike, and eel, Jarvenpaa et al.[45] observed slow excretion from the 10th to the 100th day. They found some indication of rapid loss during the second to the fourth days. They calculated elimination, including vomiting, for the first few days for protein-bound ingested methyl mercury and ionic methyl mercury. The pike lost ten percent of the former and five percent of the latter. The eels lost 17% of both. The flounder lost 33% of the protein-bound and 12% of the ionic form. Miettinen et al.[46] studied the elimination of protein-bound methyl mercury in man. In the first three to four days, six percent had been eliminated in the feces and a small amount in the urine. With increases in time, they found a less marked difference in the quantity of mercury excreted through the two routes.

Half-times of excretion have been recorded for these same species. Miettinen et al.[46] gave an average half-time of 65-75 days for either protein-bound or ionic methyl mercury in man. Aberg et al.[39] gave a similar range (70-74 days). Miettinen et al.[46] found a mean half-time of 71 days for women and 79 days for men. These latter values were not significantly different. However, other differences in response of mercurials associated with sex factor have been observed. Loutit[47] found that Pseudomonas aeruginosa with the (FP) sex factor were more resistant to $HgCl_2$. A higher concentration of mercury is present in male dogfish sharks[48] and rats[42] while male and female harbour porpoises contain the same concentration of mercury.[49] Fowler[31] observed more marked histological changes in the kidneys of female mice.

Much longer half-times were found in some aquatic species. Jarvenpaa et al.,[45] citing their earlier work, stated a half-time of 100-170 days for pike, flounder, and eel. In a later experiment, they found a half-time of 600 days for these same fish. After oral doses of protein-bound and ionic methyl mercury, they observed half-times of 780 and 700 days, respectively, for flounder, 750 and 640 for pike, 910 and 1030 for eel. Miettinen et al.[20a] studied half-times of elimination for pike and rainbow trout. Half of the ionic methyl mercury was eliminated in 94 days while 110 days was required for protein-bound methyl mercury. The proteinate vs. ionic forms showed insignificant differences in half-times.[20a,45] Jarvenpaa et al.[45] summarized by stating a half-time of approximately two years for the bulk of methyl mercury ingested by these organisms. Miettinen et al.[50] found that the half-times of excretion for pike, flounder, and perch varied from 112-490 days for different experiments. They observed a faster excretion rate for the bulk of the mercury in a mollusc (11-50 days). The half-time of excretion for the slower excretion phase in the crayfish was 144-297 days. The following half-time of elimination were recorded by Miettinen et al.:[20b] Serranus scriba, 267 days; Carcinus maenas, 400 days; Tapes decussatus, 481 days; Mytilus galloprovincialis, 1000 days. They found that the fast component of elimination comprised only 4-6% of the total mercury present for the first two species and about 20% for the last two. The resorption of methyl mercury excreted into bile may be one reason for the prolonged biological half-life of methyl mercury in animals.[51]

Friberg[52] discussed the distribution of multiple subcutaneous injections of methyl mercury. Doses were given once daily for seven days. One day after the initial series of injections, 33% of the total body content was found in the blood and 33% in the kidney. Ten percent was found in the liver, 16% in the spleen, and 2% in each of the following: cerebrum, cerebellum, and brain stem. By the 16th day, the blood concentration had fallen to half that seen one day after the injections ceased. The cerebral concentration was still 75% of the first value on the 16th day. On the 16th day after injections ceased, the cerebellum and the brain stem were reduced to 80% of the first day's value. The kidney had 90% of the first day's value, and spleen and liver had 50%. No excretion or half-time data were given.

During the passage of methyl mercury through an organism, it can be transformed to inorganic mercury. This transformation is not dependent on the bacteria inhabiting the gut of vertebrates.[53] Ostlund[38] observed biotransformation of a methyl mercurial containing both C-14 and Hg-203. By observing C-14 exhalation, he found that 4.2% of the given dose was transformed on the first day, 1.2% on the second, and 0.3-0.9% every day from the third to the tenth. Since the ratio of C-14 to Hg-203 in the body did not change, the

loss of C-14 was not the result of an exchange reaction. Norseth and Clarkson[54] observed biotransformation of methyl mercury in rats. Small amounts of inorganic mercury were found in blood plasma the first to the tenth days after injection (0.004-0.009 μg/ml). Equal amounts were found in the red cells with inorganic mercury comprised 0.2% of the mercury. The brain contained mainly organo-mercurials at all times tested. One to four percent inorganic mercury was found in the brain. Inorganic mercury increased slowly in the kidney reaching a maximum at 28-30 days. On the 16th day after injection, inorganic mercury accounted for one-half of the total mercury in the kidney and on the 30th day, 90%. In the liver, 33% of the mercury was in an inorganic form by the 25th day and 50% by the 50th day. Fifty percent of the mercury in the feces was in the inorganic form by the 10th day. With increasing time after injection, the pattern of distribution of the released mercury approached that of injected salts (accumulation in the kidney). Biotransformation is dose dependent with the percent of the original dose which is transformed decreasing with increasing dose.[55]

Norseth and Clarkson[54] stated that organic mercurials manifested different properties than inorganic because of the stability of the C-Hg bond. The slow rate of excretion in rats possibly is due to the trapping of methyl mercury in the blood cells and the slow rate of metabolism of this compound. Therefore, biotransformation of methyl mercury to inorganic mercury is important in the excretion of the former compound.[54, 55, 56]

A number of factors could influence the uptake, distribution, and excretion of methyl mercury. Studies have included the effects of ionic *vs.* protein-bound methyl mercury and dosage effects on uptake, distribution, and excretion. Ostlund[38] has studied a number of other factors such as previous exposure, sex, and activity.

Miettinen *et al.*[46] investigated the effects of different forms of methyl mercury (ionic or protein-bound) on excretion of methyl mercury. They found an average half-time of 76 days for protein-bound methyl mercury. This value is close to the range for methyl mercuric nitrate (65-75 days). Miettinen *et al.*[46] and Jarvenpaa *et al.*[45] showed insignificant differences in half-time of excretion for protein-bound and ionic methyl mercury in several aquatic vertebrates.

Several investigators have contributed to knowledge of dosage effects. Ulfvarson[57] stated that the distribution and excretion of methyl mercury is independent of dose. Piper *et al.*[41] found that the percentage distribution among organs was constant for different dosage levels. In contrast, Ostlund[38] found that distribution was not proportional to dose. The percentage of dose/gram of tissue increased in blood corpuscles with increasing dose, although plasma concentration was the same at all doses. The brain concentration was

a constant percentage of dose. Muscular tissue showed a lower percentage uptake with increasing dose as did the liver. The percentage concentration was constant over all dosage levels. Ulfvarson[57] stated that the half-time of excretion increases with decreasing dose. Ostlund[38] experimentally found the opposite. He showed that in mice the half-time of excretion increased with increasing dose and suggested that this change could have been caused by increasing inhibition of sulfhydryl enzymes at higher levels.

Ostlund[38] compared the effect of sex and swimming activity on excretion rate of methyl mercury and found no difference for mice. Miettinen et al.[46] found no effect of sex on excretion of methyl mercury for man. In addition, Ostlund[38] found that trace doses of radioactive mercury administered after large doses of non-radioactive methyl mercury distributed in the same way as doses of trace amounts. He suggested that as the larger dose was excreted, the first sites to which it was bound were made available to the trace dose administered later. Increasing time of exposure results in increased concentration of methyl mercury; therefore, increase in mercury concentration with increasing age or size has been observed in many organisms.[58, 59, 60, 61, 62]

Dimethyl Mercury

Ostlund[38] studied the distribution of dimethyl mercury in mice. He found no difference due to route of administration (inhalation or intravenous injection). In both cases, he observed a rapid reduction in blood concentration with simultaneous deposition of dimethyl mercury in fat (within five minutes after administration). Five minutes after administration, the bronchi and nasal mucosa were high in mercury concentration. Moderate concentrations of dimethyl mercury were observed in the liver and kidney, though the renal cortex had a high mercury concentration. A concentration similar to that in the blood was seen in the spleen, lungs (except bronchi), and intestines. Moderate uptake was observed in the salivary glands after five minutes. The mercury concentration varied for different parts of the central nervous system after five minutes. Mercury content of the cerebral cortex and cerebellum were lower than that in the blood. After five minutes, most of the mercury present was in the form of dimethyl mercury. The brain stem and medulla oblongata showed concentrations similar to that of the blood. The concentrations of the hypophysis and the spinal cord were similar to that of the liver.

At 20 minutes, the mercury concentration of the blood was low and the concentration in the fat, high. Concentration of mercury was increasing in the liver, kidney, and the red pulp of the spleen. The gall bladder showed the same concentration as the liver. The

concentration in the bone marrow was lower than in the blood. A high concentration of mercury was observed in the nasal and oral mucosa, the pharynx, esophagus, and pancreas. After 20 minutes, Ostlund[38] found that all tissues except the fat deposits, brown fat, and the central nervous system contained mercury in the form of a non-volatile metabolite. The fat tissues contained only dimethyl mercury.

At one hour, Ostlund[38] found a decrease in the total body concentration although the kidney, liver, and adrenal cortex concentrations had increased. The mercury concentration in the kidney, gall bladder, liver, and fat were the same. The concentration in the fat had decreased in relation to the blood. The nasal, pharyngeal, oral, and esophageal mucosa concentrations remained unchanged while the gastric and intestinal mucosas showed a marked accumulation of mercury. An increase in mercury concentration was observed in the central nervous system and hypophysis. All organs contained non-volatile metabolites of dimethyl mercury.

After four hours, the mercury content of the fat remained higher than that of the blood. The bronchi, liver, kidneys, and adrenal cortex contained higher concentrations than the fat. A small concentration was observed in the muscles. A few areas of the intestines showed a high mercury content while the remainder of them showed a concentration comparable to the blood. All organs contained non-volatile metabolites except the fat and central nervous system which still contained mercury in the dimethyl form.

After 16 hours, the distribution of dimethyl mercury and non-volatile metabolites was the same as after four hours. The highest concentrations were observed in the kidney, liver, and nasal mucosa. The muscles, intestines, spleen, and lungs showed concentrations of mercury comparable to the blood. No mercury accumulation was seen in the bronchi. The concentration in the upper digestive tract had decreased by 16 hours, and a moderate mercury uptake was observed in the lens and hypophysis. The fat tissue and tissue of the central nervous system both showed mercury concentrations less than that found in the blood. No mercury was detected in the connective tissue or compact bone.

By 24 hours, the nasal mucosa and inner cortex of the kidney were only tissues containing large amounts of mercury. Differences in the mercury concentration in most structures had disappeared. Less than the average concentration was seen in the central nervous system and fat. After four days, the central nervous system concentration had increased, and uptake by the hair follicles was observed. Uptake in the basal part of the hair occurred by 16 days. The mercury concentration of the central nervous system was the same as that of the blood by this time.

Ostlund[38] also described fetal uptake in pregnant mice. As early as five minutes after injection or inhalation, he observed moderate mercury uptake in the ovaries and oviducts though no fetal uptake was seen. The placenta showed a mercury concentration comparable to that of the blood. By one hour, the fetuses showed marked mercury uptake in their nasal, oral and esophageal mucosa, and bronchi. Moderate uptake was observed in the liver. Only the placenta contained dimethyl mercury as the sole mercury form. Fetal tissues contained non-volatile metabolites of dimethyl mercury. The mercury concentration of the fetuses was approximately the same as the maternal blood by 16 hours. No accumulation was observed in the bronchi, nasal, oral or pharyngeal mucosae though the lens showed marked uptake.

Ostlund[38] also measured excretion of injected and inhaled dimethyl mercury. He found two phases in the excretion rate curve, a rapid phase during the first six hours and a slow phase. The rapid phase corresponded to the time required for exhalation of dimethyl mercury. Ten percent of the body burden of mercury remained after the rapid phase of excretion was completed. The half-time of the slowly excreted metabolite was 9.5 days. The half-life of a similar dose of methyl mercury is 8.9 days. Ostlund suggested that this slowly excreted metabolite is methyl mercury. A rapid excretion phase of six hours was also observed with inhalation as the route of administration. The half-time of elimination of the slow phase was seven days. Ostlund concluded that the excretion rate is the same for the two routes of entry.

Ostlund[38] described the mercury present in mice after administration of dimethyl mercury as volatile or non-volatile metabolites. He showed that the volatile exhaled portion was dimethyl mercury. The even distribution pattern of the non-volatile portion gave support to the hypothesis that the non-volatile portion is methyl mercury. Thin-layer chromatography confirmed this hypothesis. The only organic metabolite of dimethyl mercury is methyl mercury.

Phenyl Mercury

Several investigations of the distribution of phenyl mercurials after single intravenous injections have been conducted. Swensson *et al.*[35] determined the mercury content of the blood, brain, liver, and kidneys at specified times after injection into rats. After three hours, the mercury content of the blood was the highest of the four analyzed but steadily declined during the following hours. The kidney contained the second highest concentration though it was much lower than the blood. The brain and liver contained the same amount of phenyl mercury (less than ten percent of the blood concentration). By the end of 24 hours, the kidney content had risen sharply, increasing to 125 times the 3-hour value. The liver content was 10 times its 3-hour value.

After four days the kidney content had fallen to one-fourth its 24-hour value and the liver content had declined to less than one-sixth of its former value. The blood had decreased to one-third its previous content, but the brain content remained the same. After 16 days the mercury content of all tissues measured remained the same with the exception of the blood content which had declined sharply. After 32 days only small amounts of mercury were found in all tissues.

Swensson et al.[35] also studied the mercury distribution in dogs at four hours after intravenous injection. The organs tested included the liver, kidney, brain, cerebellum and colon. The kidney contained the highest concentration, and the liver and colon contained the next highest concentrations. Each contained approximately one-ninth the concentration in the kidney. The brain and cerebellum contained the lowest amount. Together they had accumulated approximately one-fortieth of the concentration found in the kidney. In addition, Swensson et al.[35] found that 4.3% of the mercury was excreted in the urine within four hours after injection.

Swensson and Ulfvarson[36] investigated the distribution of phenyl mercury in poultry. They measured mercury content of the brain, kidney, liver, muscles, and blood 10 and 20 days after injection. After 10 days they found the highest concentration in the kidneys and liver. The muscle contained about one-third that found in the kidneys or liver. The brain contained even less, and the blood showed the lowest concentration. After 20 days, the kidney had a greater concentration than the liver, and the brain contained over three times more mercury than the muscle. In addition, they found that the distribution between different organs was constant following injection with the exception of the kidney content which increased in relation to the other organs with time although its absolute mercury content decreased. They observed a similar distribution pattern in dogs.

Measurements of excretion of intravenously-injected phenyl mercury revealed that 20% of phenyl mercury hydroxide remained in the body 10 days after injection.[36] The excretion data for phenyl mercury fit a hyperbolic function with a rate constant of 1.1×10^{-4} day^{-1} (μg mercury/kg body weight)$^{-1}$.

Ulfvarson[33] studied the distribution of single subcutaneous injections of phenyl mercury in rats. Three days after injection he found the highest concentration of mercury in the kidneys and the second highest in the liver. The blood, muscle, and brain were low in mercury concentration.

Intramuscular injection of phenyl mercury in birds was studied by Miller et al.[63] They found that the total mercury in the liver

remained constant for 96 hours. The total mercury in the kidney increased for 24 hours and then remained constant for 96 hours. After three hours the phenyl mercury content of the liver declined, and after 12 hours the phenyl mercury content of the kidney decreased. Only about half of the kidney mercury was in the form of phenyl mercury.

Several researchers have studied dosage effects of phenyl mercury. Miller et al.[63] found that liver and kidney concentrations were proportional to dose in birds. Ulfvarson[57] found some changes in distribution in rats when the dose was increased by a factor of ten. He found that excretion rate was only slightly affected. The blood concentration was much higher than expected while the liver contained less than anticipated. By the 24th day, all mice showed a lower mercury concentration than expected. The increased rate of excretion reflects a saturation phenomenon at the higher dosage.[33]

Methoxyethyl Mercury

Ulfvarson[33] described the distribution of subcutaneously injected methoxyethyl mercury in rats. Three days after injection he found the highest concentrations in the kidneys and liver. The blood and muscles showed similar concentrations and were several times lower in mercury concentration. The brain only showed a trace of mercury. Ten to 20 days after intravenous injection in poultry, Swensson and Ulfvarson[36] found that kidney concentration was higher than liver concentration but this difference appeared to decrease with time. The brain concentration was the third highest and muscle, fourth. The mercury concentration of the blood was low.

Swensson and Ulfvarson[36] found that 90% of the methoxyethyl mercury was excreted by the 10th day after intravenous injection in poultry. They described excretion vs. time as a hyperbolic function. They found an excretion rate of 7.1×10^{-5} day^{-1} (μg mercury/kg body weight)$^{-1}$.

Ethyl Mercury

Miller et al.[64] studied ethyl mercury distribution after oral doses and intramuscular injections in poultry. They investigated the distribution of mercury and the percent remaining as ethyl mercury. The liver consistently retained the highest concentration of mercury, the kidney second, and the blood lowest. Ethyl mercury was present in the liver for 21 days though the percentage of total mercury in the form of ethyl mercury declined after 10 days. Ethyl mercury was present in the kidney for 14 days though it declined after two days. It was present in the blood seven days. The percent as ethyl mercury declined after two days. Miller et al.[64] found similar distribution and metabolism of ethyl mercury after oral administration. However,

ethyl mercury was detected in the tissues as such for slightly longer periods of time. In addition, they showed that rats injected intramuscularly contain more ethyl mercury in the kidney and blood and less in the liver. They hypothesized that the liver was the main detoxification reservoir for poultry and the kidney for mice.

Rucker and Amend[65] studied the retention of ethyl mercury in fish exposed to mercury in the water. The mercury content of the gill of rainbow trout rose rapidly the first two hours after a single exposure and declined the next 24. After two weeks it slowly reached a background level. The blood level rose as the gill concentration declined and reached a maximum at 32 hours. The blood level began to decline by the third day and attained a background level after six to eight weeks. The mercury level of the liver rose gradually and reached a peak by the third day. It dropped slowly and did not approach background level until the 20th week after exposure. The mercury level in the kidneys rose during the first 24 hours, then declined slightly during the next seven days. It reached its maximum at three weeks and declined slowly thereafter.

Rucker and Amend[65] also investigated the distribution in trout after multiple exposures to ethyl mercury chloride. The mercury level in the gills was always low before daily exposure and high afterwards. The blood level showed a steady increase through the 10th day of exposure. The kidney and liver steadily accumulated mercury. Their concentrations remained lower than that of the blood, but they still accumulated mercury through the last treatment. During twelve weekly exposures, they found that the blood level increased through the 10th week and then declined. It did not return to a background level until 17 weeks after treatment stopped. The liver mercury content rose as long as exposure continued. After discontinuation of the treatment, the level declined; however, mercury was still present the 33rd week after exposures had ended. The kidneys showed a steady increase during the first three weeks, then a sharp increase. Mercury content of the kidneys continued to increase until one week after exposure had ended. The content then declined but was still high the 44th week after exposure. The mercury content of the muscle was always lowest. It reached a maximum during the 12th week and declined to background by the 17th week.

Mercuric Salts

Friberg et al.[66] described the distribution of subcutaneously injected mercuric chloride in rabbits. Highest concentration was in the kidney and the second highest was in the liver. Only small amounts of mercury were found in the brain. Swensson et al.[35] found that mercury was rapidly fixed to the kidneys of rats reaching a maximum concentration 24 hours after injection. Swensson and Ulfvarson[36] showed that 40% of the intravenously injected mercuric

nitrate was retained in poultry 10 days after injection. Their excretion data fit a hyperbolic curve with a rate constant of 1.9×10^{-4} day^{-1} (μg mercury/kg body weight)$^{-1}$. Swensson et al.[35] showed that 2.3% of intravenously injected mercuric nitrate was excreted within four hours after injection. Large amounts were also accumulated in the colon indicating appreciable fecal excretion.

Though it appears that the two routes of administration (intravenous and subcutaneous injections) give rise to the same distribution pattern, Miyama et al.[67] has shown some differences between these two routes of injection. He sacrificed rabbits 4 and 24 hours after injection. The mercury content of the brain was always higher for subcutaneously injected animals. At four hours the amount of mercury in the brain of subcutaneously injected rabbits was twice that found in animals receiving intravenous injection. In contrast, the mercury content of the liver was always lower in subcutaneously injected rabbits. Except for the fourth hour, the mercury concentration of the kidney was higher in animals receiving subcutaneous injections. Mercury excretion in the urine was higher in animals intravenously injected though no significant difference in fecal mercury was shown for the two routes of injection.

With increasing dose of intravenously injected mercuric nitrate, Ulfvarson[33,57] observed a saturation phenomenon in rats. The rate of excretion was probably increased with increasing dose. Body components contained less mercury than expected at increasing dosage levels. Thus, relative distribution and excretion were influenced by the size of dose.

Metallic Mercury

Several researchers have become interested in the effects of exposure to mercury vapor since this route of administration is common for individuals industrially exposed. Kudsk[68] suggested that uptake occurs at the alveoli of the lungs by simple diffusion. Berlin et al.,[69] in investigating the distribution of mercury vapor in rats, rabbits, and monkeys, found no difference in species. The body distribution was approximately the same as that found for injected mercuric salts; however, the mercury content of the brain of vapor-exposed animals was 10 times that of animals exposed to the mercuric salt. They emphasized that differences in brain uptake are not due to the exchange in the alveoli but in differences in blood cell/plasma ratios for the two mercurial forms. No difference in rate of elimination from the body was observed for the two forms.

Magos[70] studied the intravenous injection of metallic mercury and mercuric chloride in rats. He found that loss by exhalation started immediately and lasted 15 seconds. Much less mercuric chloride was lost than injected mercury during this period of time. More metallic

mercury than mercuric ions was found in the lungs, brain, and heart 30 seconds after exposure. Only 6% of the injected dose of metallic mercury was retained in the blood while 45% of injected salt was retained. Magos[71] suggested that this difference in blood content was influenced more by diffusion than by oxidation of metallic mercury. The ratio of brain mercury content/blood mercury content was 10 times higher for metallic mercury.[70] Thirty seconds after injection, body and blood content were approximately equal for metallic mercury while 11 times as much mercuric chloride was found in the blood as outside the blood.[70] After 30 seconds the mercury content of the blood fell slowly. By this time both injected mercury and mercuric ion were in the same oxidized state.

Magos[72] discussed retention of mercury vapor in white mice at different dose levels. The uptake was dependent on degree of exposure for all organs examined. Twenty-four hours after exposure, the kidneys showed the highest content (12.36% of the body burden). The fur and skin were slightly lower in content (11.62%). The liver contained 6.02% and the lungs, 4.62% of the total concentration. The brain contained 2.84% of the body burden. The smallest amounts of mercury were detected in the heart (0.83%) and blood (0.42%). The heart and lungs lost mercury more rapidly than the body average while the blood and brain lost it more slowly. After eight days the brain and kidney had almost equal percentages of the body burden.

In summary, different routes of administration give similar distribution and excretion patterns for each mercurial with the exception of metallic mercury. Birds, dogs, rabbits, rats, mice, and man also show similar distribution and excretion patterns for specific mercurials though the main reservoirs of rats and poultry are different for some mercurials. However, some aquatic vertebrates exhibited markedly different excretion rates though among different fishes, the excretion rate was similar. Phylogenetically related species appear to follow similar patterns of methyl mercury elimination with some dependency on mode of entry and temperature.[20] In addition, dosage levels appear to influence excretion rates for most mercurials. Though the distribution pattern is not changed with increased dose, the percentage retained in each organ is frequently less than anticipated at higher dosage levels. The last major factor influencing uptake, distribution, and excretion of mercurials is the type of mercurial. This is perhaps more significant than other factors since uptake, distribution, and excretion are all influenced by the type of mercurial. The significance of this factor necessitates further discussion.

Comparison of Mercurials

Several basic differences in distribution of mercurials are apparent. Friberg[52] compared an inorganic mercury salt to methyl mercury. He

found that more mercury was accumulated in the methyl form. The blood concentration was 100 times greater than for inorganic mercury.[52] Ulfvarson[33] found greater retention of methyl mercury than methoxyethyl mercury and mercuric nitrate in the brain. Swensson et al.[32,35] also found a higher content of organic mercurials in the blood. They found greater blood retention with alkyl than phenyl mercurials. The liver showed greater accumulation with alkyl mercurials.[35] Renal values were twice as high as for the inorganic form. Friberg[52] found that rats given inorganic mercury excreted twice as much in the feces and more than 20 times as much in the urine as those given methyl mercury. Though the percentage loss from the parts of the brain were the same for the two mercury compounds, mercuric chloride content diminished more rapidly than methyl mercury. Rates of loss from the liver and spleen were greater for inorganic mercury although the difference was not significant.

Swensson and Ulfvarson[36] compared injections of phenyl mercury, methyl mercury, mercuric chloride, and methoxyethyl mercury in poultry. The brain content in all cases was 5 to 10 times higher than the blood content. The brain concentration for methyl mercury was highest of all. They found that the relative distribution among the different organs remained constant except for the kidney. The kidney concentration of phenyl mercury decreased with time but, in relation to other organs, its concentration increased. The relationship was found to be variable with mercuric nitrate. Swensson and Ulfvarson[36] stated that the general distribution pattern revealed a uniform distribution for methyl mercury. All other mercurials showed low blood and muscle concentration with high concentration in the kidney and liver. Testing excretion rates, they found that methoxyethyl mercury has the fastest (10% remaining after 10 days), phenyl mercury the next (20% remaining), mercuric nitrate (40% remaining) next, and methyl mercury the slowest (80% remaining). As previously mentioned, Swensson and Ulfvarson[36] characterized the excretion vs. time curve as an exponential function for methyl mercury and as a hyperbolic function for all others.

Friberg et al.[66] also noted a similarity between phenyl mercury and an inorganic mercuric salt. For both compounds, they found the greatest concentration in the kidneys with the liver containing the second highest concentration. Only small amounts were found in the brain. The concentrations for both compounds were of the same order of magnitude. Mercuric nitrate concentration in the kidney provided an exception to this distribution pattern. On day 6 its content was higher than day 1. By day 40, it was 60% of the day one concentration. Readings successively fell for phenyl mercury and were about 20% of the day 1 concentration by day 40. Friberg et al.[66] found that distribution patterns within organs were similar for the two compounds except for the liver. Phenyl mercury showed even distribution within the liver while mercuric nitrate showed concentration

in areas of connective tissue. In general, Friberg stated that deposition was similar but that the inorganic form disappeared more slowly from the kidney.

Swensson et al.[35] compared phenyl mercury, methyl mercury, and mercuric nitrate. They found that mercuric nitrate had the greatest tendency to become fixed in the kidneys in rats. Phenyl mercury accumulated in lower concentration. Methyl mercury accumulated least and was more evenly distributed among organs. They stated that 10 times as much methyl mercury as mercuric nitrate accumulated in the brain and 2.5 times as much methyl mercury as phenyl mercury. Blood content immediately after injection was greater for mercuric nitrate, phenyl mercury was second, and methyl mercury, least. In accordance with data previously presented, the excretion rate was very slow for methyl mercury. During the first four hours, 4.3% of the phenyl mercury was excreted, 2.3% of mercuric nitrate, and only 0.33% (maximum found) of methyl mercury.

Suzuki et al.[37] compared placental transfer of different mercurials. They found the largest transfer with methyl mercury and the least with phenyl mercury. Mercuric chloride was transferred in a quantity twice that of phenyl mercuric acetate but only one-tenth that of methyl mercuric acetate. Methyl mercury was fairly uniformly distributed within the fetus and placenta though a lesser amount was found in the amniotic membrane. Mercuric chloride and phenyl mercuric acetate were retained uniformly within the fetus but in higher concentrations in the amniotic membrane and placenta. The placenta therefore appeared to provide a barrier to the passage of mercuric chloride and phenyl mercuric acetate but not to methyl mercuric acetate. The ratio of the mercury content of the maternal blood:placenta:fetus was as follows: mercuric chloride, 1:19:0.4; phenyl mercuric acetate, 1:4.5:0.3; methyl mercuric acetate, 1:19:2.1. There appeared to be a difference in binding and release of mercurial from the placenta determined by chemical structure.[37] Suzuki et al.[37] suggested that water solubility may play some part in the retention on the amniotic membrane.

A unique type of biotransformation has been observed with some mercurials-in vivo methylation. Kiwimae et al.[73] investigated mercury transfer to eggs and methylation. They found that phenyl mercuric hydroxide, mercuric nitrate, and methoxyethyl mercuric hydroxide were all partially transformed to methyl mercury in hens. The methylation process, however, was not rapid or complete. The mercury concentration in the hen eggs was influenced by rate of excretion and the mercury compound. Initially, the total mercury concentration in the eggs increased rapidly. After one or two months the rate of increase in the eggs was only moderate. The egg concentration was dependent on the level in the food. The order of accumulation of mercurial from highest to lowest was as follows:

methyl mercury, phenyl mercury, and methoxyethyl mercury. The concentration between yolk and white also varied with the mercurial. Only methyl mercury showed a higher concentration in the white. With methyl mercuric hydroxide, the influence of increase in dose effect was linear, a four-fold increase in dose yielded a four-fold increase in mercury concentration of the white. With mercuric nitrate and phenyl mercuric hydroxide, the dose level only slightly affected the concentration in the white. The content of the white doubled with a four-fold increase in dose for methoxyethyl mercury. The yolk showed a four-fold increase in mercury concentration for a four-fold increase in dose for all mercurials tested. The percentage of mercury as methyl mercury in the whites one week after feeding began was 81-94%. This remained constant during feeding but fell rapidly after feeding stopped. For other compounds, the mercury content of the whites was less affected by dose than the yolk. No particular equilibrium between whites and yolks appeared to exist. Methyl mercury was the primary constituent of the whites from hens given any one of the compounds. The concentration varied with the compound given but was lowest when methyl mercury was given. Greater than half of all the compounds given were converted to methyl mercury by the time they reached the egg. Methyl mercury was also found in the organs. Greater than 50% of the mercury in the muscle and blood was methyl mercury. Only small percentages of methyl mercury were found in the kidney and liver for all mercurials tested. Microbial methylation by specific bacteria and fungi or in mud sediments has been investigated by several researchers.[74, 75, 76, 77, 78, 79] Genetic connection between methionine biosynthesis and mercury resistance has been demonstrated.[80] Lander[76] has shown that methyl mercury synthesis involves several steps in methionine biosynthesis and provides a mechanism for the occurrence of methyl mercury products in organisms not exposed to methyl mercury.

Ulfvarson[57] investigated the difference in dosage effects among mercury compounds. Only with methyl mercury was the relative distribution and excretion of mercury unaffected by dose. With methoxyethyl mercury an increase in dose by a factor of 10 resulted in organ concentration lower than expected. Mercuric nitrate also showed lower values than expected. Therefore, all but methyl mercury showed a saturation phenomenon.

In summary, all parameters of mercurial distribution (i.e., organ retention, placental transfer, egg transfer, etc.) revealed that the most marked difference of mercurials is between methyl mercury and other mercurials. Ethyl mercury has been placed in the same category as methyl mercury.[37] Though quantitative differences in distribution pattern can be shown between mercuric nitrate, phenyl mercury, and methoxyethyl mercury, they all show similar characteristics. For example, dosage response shows a similar pattern, the excretion pattern follows the same curve, and placental transfer is blocked. Thus, alkyl mercurials stand out among other mercurials and markedly influence uptake, distribution, and excretion characteristics.

SECTION IV

ORGAN AND TISSUE EFFECTS

Studies of the effects of mercurials on tissues resulted primarily from the Minimata incident. Subsequent investigations on animals other than man have given rise to knowledge of the intra-organ distribution and physiological changes at the tissue level such as changes in tissue respiration. Since marked neurological signs have been observed, the central nervous system has been the focus of many investigations. The blood has also been extensively studied in order to elucidate differences in transport of different mercurials. The liver, kidneys, gill, heart muscle, and eye lens have also been included in tissue studies of the effects of mercurials.

CENTRAL NERVOUS SYSTEM

Berlin and Ullberg[34] found that the distribution of methyl mercury following single intravenous injections in mice was fairly uniform within the brain. This differed from the heterogeneous distribution resulting from the injection of mercuric salts. They found that the hippocampus and gray matter of the cerebellum take up the most methyl mercury. As in inorganic mercury poisoning, the gray substance has a higher concentration than the white matter. Aberg et al.[39] found a localization in the cerebellum in man exposed to low methyl mercury levels. Yoshino et al.[81] found that high mercury in the cerebellum was inconsistent. He found highest mercury content in the calcarine areas. Piper et al.[41] found the highest concentration of methyl mercury in the cerebrum and the lowest in the spinal cord of pigs. After a single oral administration, Berlin and Ullberg[34] found that distribution in the cerebellum, brainstem, and spinal cord of mice was fairly constant from days 7-35. The concentration in the cerebrum varied more than other tissues with an apparent preferential retention up to the 13th day. After subcutaneous and intravenous injections of mercury sublimate in rabbits, Miyama et al.[67] observed the highest mercury concentration in the brainstem for both routes. The cerebellum was generally second in concentration. Either the cerebral cortex or hippocampus was lowest in mercury concentration.

Berlin et al.[69] studying several mammals (rats, rabbits, monkeys) compared distribution in the brain of vapor-exposed vs. injected mercury salts. In mammals exposed to mercury vapor, they found more mercury in the gray matter than the white matter. The nucleus dentatus in the cerebellum, nucleus olivarius inferior in the brain stem, the choroid plexus, and the nucleus subthalamicus all showed marked mercury uptake. The collicus superior also appeared to show accumulation. In the cerebral cortex, different uptake was observed

in different cortical layers with the ganglionic higher than others. In the cerebellar cortex, the Purkinje and granular cells appeared to accumulate more mercury than the molecular layer. The periphery of the dense zone showed higher accumulation. Some differences in distribution due to route of administration were observed. The choroid plexus and pia mater were higher when compared to the rest of the brain in injected animals. The area postrema showed greater accumulation than the rest of the brain for injected but not for vapor exposed animals.

Extensive histological examinations of human tissue have been made by several investigators. Tokuomi[2] stated that the distinct general features of methyl mercurialism were diffuse encephalpathy with cerebellar and cerebral cortex involvement and effects of pyramidal and extrapyramidal tracts with disturbances of peripheral nerves. His macroscopic observations revealed cerebral swelling and turbidity of the meninges in acute cases. Chronic cases showed atrophy of the brain with increase in surrounding fluid. Convolutional atrophy was especially apparent around the medial aspects of the occipital lobes and the anterior ends of both calcarine fissures. The cerebellum was also atrophied and the gray matter thin. Kurland et al.[8] observed scattered punctate hemorrhages in addition to the characteristics reported by Tokuomi.[2] McAlpine and Araki[9] also reported edema and occasional hemorrhages. Hunter and Russell[82] macroscopically observed a few flecks of atheroma in the main cerebral arteries. In addition to the features reported above, Hunter and Russell found that the left optic nerve was slightly divided at the chiasma and the right was slightly flattened. They found occasional small foci of atrophy in the cortex of the Rolandic area and the frontal and temporal lobes. The normal cortical striation of Gennari were absent. They also described atrophy of folia in the depths of the sulci in the lateral lobes of the cerebellum. The flocculli and dentate nuclei were not affected.

Fimreite and Karstad[25] observed different characteristics after acute exposure of red-tailed hawks to methyl mercury. The primary site of damage was the spinal cord instead of the brain. They observed swelling of the axons and myelin of the myelinated nerves of the spinal cord. The nerve root and dorsal root ganglia were infiltrated with heterophils. Klein et al.[22] observed similar characteristics in the sciatic nerve. Lesions were greater in the spinal cord and reduced cerebrally.[25]

Microscopic examination of brain tissue from humans whose death resulted from methyl mercurialism revealed many signs of damage. Hunter and Russell's[82] microscopic examination of the cerebrum showed that the cortex of the area striata was atrophied in both hemispheres. They attributed constriction of the visual field to gross atrophy of the area striata. The atrophy was greatest about

the anterior end of calcarine fissures.[2,82] Yoshino[81a] found that the calcarine fissure was also the most prominent area of effect in dogs. Hunter and Russell[82] found a great loss of neurons with the most severely affected showing only gliosis. The remaining neurons were small and many were distorted. In addition, they reported no fat in the granule cells in the cortex or white matter. The subcortical white matter was also reduced. They found that the oligodendroglial cells were in stages of acute swelling. Hunter and Russell[82] reported for both macro- and microscopic investigations that the severity of atrophy and cell destruction in the area striata decreased with approach to the occipital pole. They observed the foci of cortical atrophy in the left precentral, the right post-central, and left superior temporal gyri. The hypothalmus, brain stem and basal ganglia were not affected. In contrast, McAlpine and Araki[9] and Kurland et al.[8] reported changes of varying degrees in the basal ganglia, brainstem, and hypothalmus.

Several workers reported similar changes in the cerebellum. Hunter and Russell[82] observed a loss of cells in the granular layer. The Purkinje cells were spared and the molecular layer narrowed. Some gliosis was observed in the molecular layer. The changes were most marked in the depths of the sulci. Yoshino[81] and Kim[83] found similar changes in dogs and mylinating cultures, respectively. Only Hunter and Russell[82] described changes in Purkinje cell morphology. They found some lay abnormally high in the molecular layer with the main dendrites oblique, horizontal or directed towards the depths of the cortex. Basket and climbing fibers were absent in severely affected cells. Stellate bodies formed at the terminal ends of fibers. They attributed gross ataxia to these alterations in the cerebellar cortex.

Tokuomi[2] characterized the histological changes in three groups. First, regressive changes included the conspicuous changes in the cerebellum and calcarine cortex revealed by cell loss or spongy appearance of the cortex and white matter in severe cases. Second, progressive changes included the proliferation of glial cells. The third group of changes included disturbances of circulation characterized by hemorrhages in the gray and white matter, perivascular edema, and cystic dilation around blood vessels.

Lindahl and Hell[84] investigated a functional parameter of brain damage--respiration of brain slices. They exposed fish to 0.3×10^{-5} M phenyl mercury. A small inhibition of respiration was observed in brain slices when succinate was provided as substrate.

KIDNEY

Berlin and Ullberg[34] found early accumulation of methyl mercury and mercuric chloride in the renal cortex in mice. Friberg et al.[66] made

a similar observation for phenyl mercury and mercuric chloride in rabbits.

Histological observations after death due to methyl mercurialism revealed ishemic atrophy resulting from fatty and hyaline degeneration of the cortical arterioles.[82] In addition, fatty degeneration affected renal tubules, especially the loops of Henle in the renal medulla.[82] Klein et al.[22] found degeneration of the distal convoluted tubule. Tokuomi[2] also observed fatty degeneration of parenchymatous cells. Johnson and Koumides[14] and Klein et al.[22] observed epithelial necrosis of the renal proximal convoluted tubules after metallic mercury injection methyl mercury ingestion, respectively. The tubules contained eosinophilic debris. Spherical masses in the lumen of tubules containing ribosomes, smooth endoplasmic reticulum, cytoplasm, and sometimes a microbody have been observed in mice exposed to methyl mercury.[31] Kanzantis et al.[17] reported similar results. Johnson and Koumides[14] observed exudate around many glomeruli and the distal tubules contained altered red cells. Glomeruli of mice studied by Fowler[31] were normal. Kanzantis et al.[17] and Klein et al.[22] found schlerotic changes in some glomerili. Their histochemical analysis revealed an abundance of lipids in the proximal convoluted tubule epithelium. Areas of infiltration with round cells were found in the interstitium. Kanzantis et al.[17] reported an absence or suppression of alkaline phosphatase and abnormal succinate dehydrogenase distribution. They observed no enzyme activity in the proximal convoluted tubule though some was observed in the distal convoluted tubules and ascending loops of Henle.

Conn et al.[85] attempted to explain the cause of renal failure. They stated that changes in renal circulation were the basic cause of acute renal insufficiency. They questioned whether persistent ishemia was responsible for the development of renal insufficiency or whether intrarenal blood shunts, which may operate during periods of renal inadequacy, play a role in the development or continuation of renal insufficiency. In tests with dogs, they found that the renal circulatory bed ranged from normal in dogs with normal amounts of urine to 40-50% of normal in anuric dogs. Intrarenal distribution of blood flow was normal. Oxygen consumption and para-amino hippurate were reduced in all animals showing reduced urine flow. Tubular degeneration and necrosis were observed. Conn et al.[85] stated that reduction in para-amino hippurate and oxygen consumption were closely correlated with these morphological changes. However, oliguria or anuria can be better correlated with renal circulatory impairment. They found no evidence of intrarenal shunts. They stated that the change in urine flow is dependent on renal ishemia and nephron damage.

LIVER

Friberg et al.[86] reported that mercuric salts were accumulated unevenly in the liver with greater localization around connective

tissue, bile ducts, and the portal veins. Phenyl mercury showed a more even distribution but with more accumulation around the portal veins. Berlin and Ullberg[34] found uniform liver distribution with methyl mercury.

Arrhenius[86] studied the effects of organic mercury compounds on the detoxification mechanism of the liver. He described a detoxification system in which the first step was oxygenation of dimethyl aniline yielding dimethylaniline-n-oxide as an intermediate. In the second step, oxygen was transferred to one of the methyl groups yielding formaldehyde and monomethyl aniline. He found that 1.0 mM organic mercury inhibits detoxification greater than 80%. 0.10 mM organic mercury yields preferential inhibition of the second step. Leakage products are larger when this preferential effect is observed. The significance of this type of mercurial effect comes from the potentially hazardous effect of these leakage products.

Lindahl and Hell[84] measured some functional parameters of mercurial effect on liver slices. They found that oxygen consumption increased 15% when fish were exposed to 1 mg/L for 40 minutes. In addition, they found that the glycolytic rate was inhibited under both aerobic and anaerobic conditions.

HEART

Few observations of the effects of mercurials on heart muscle have been made. Johnson and Koumides[14] found many foci of inflammatory cells and necrosis of myocardial fibers in a human subject who had died from an injection of metallic mercury. Hunter and Russell[82] reported subendocardial fibrosis replacing muscle in individuals suffering from methyl mercurialism. Malek et al.[87] observed rapid accumulation and long term retention in ishemic heart muscle although the same did not occur in healthy heart tissue exposed to a mercurial. They also observed a rise in the heart fibrillation threshold in ishemic tissue resulting from application of a mercury fluorescein derivative. Granular changes have been observed in the cytoplasm of the myofibrils of the heart and in smooth muscle cells of some blood vessels.[25]

BLOOD

A marked difference in the distribution of different mercurials between blood and blood plasma has been observed. Swensson et al.[35] studied the distribution of methyl mercury and inorganic mercury in the blood. They found a plasma to whole blood mercury content ratio of 0.10 for methyl mercury and 0.80 for inorganic compounds. Suzuki et al.[87] showed a similar trend for alkyl vs. inorganic mercurials. The mean ratio for workers exposed to metallic mercury was 1.3. A ratio of 14-27 was found for subjects exposed to alkyl mercury and

5.92-11 for those exposed to protein-bound methyl mercury. When the ratio of mercury concentration in blood components is calculated as red cell to plasma mercury content, a ratio of 0.625 results from administration of metallic mercury and 27-53 is found in subjects exposed to alkyl mercurials. Berlin et al.[69] reported higher red cell to plasma mercury concentrations in vapor exposed mammals than in mammals injected intravenously with a mercuric salt. Sixty-seven to eighty-four percent of the mercury was bound in red cells in the former case while the latter only showed 25-31%. No differences in blood binding of mercuric salts was observed for subcutaneous vs. intravenous injection.[67]

Kudsk[68] observed a high rate of mercury vapor uptake by the plasma during the first hour after exposure. Fifty percent of the total mercury in the blood is located in the plasma during the first hour. During subsequent hours the plasma uptake is only 10-15% of that of the whole blood. Therefore, 85-90% of the mercury taken up after the first hour is probably oxidized in the erythrocytes.[68] Magos[72] described two phases of uptake of mercury vapor-a fast phase followed by a slow, steady increase. The percentage absorbed was linearly related to hemoglobin concentration.

The difference in mercury uptake in erythrocytes for different mercurials appears to be due to the stability of alkyl mercurials bound to hemoglobin. Takeda et al.[89] found that ethyl mercury was bound to hemoglobin as ethyl mercuric cysteine by mercaptide linkages. Within 30 minutes after exposure, most of the mercury was bound inside the erthyrocytes. Once combined, release was difficult because of the high stability of the bond. Suzuki et al.[90] suggested a similar explanation for the elevation of alkyl mercury in red cells of the umbilical cord and fetus over that of the maternal blood.

Clarkson[91] and Kudsk[92] discussed factors that could influence the uptake of mercury vapor. Clarkson[91] found that an increase in oxygen accelerated mercury uptake and stated that the vapor was being oxidized to the ionic form. After studying the influence of a number of compounds on mercury vapor uptake, Kudsk[92] found a possible relationship between uptake, oxidation, and the coupled glutathione and carbohydrate metabolism.

In addition to investigations of uptake and distribution within the blood, some researchers have been interested in the effects of mercurials on blood components. Lindahl and Hell[84] found hemolysis of fish erythrocytes. The half-time of hemolysis for fish incubated in 0.5×10^{-4} M phenyl mercury at 20 C was 55 minutes. No hemolysis was observed at 0.3×10^{-5} M. Benesch and Benesch[93] investigated half-times of hemolysis for Salyrgan, a diuretic, and phenyl mercury. A half-time of hemolysis of 103 minutes was found for the former and

28 for the latter. Hemolytic action of alkyl mercurials varies in the following way from most active to least: n-butyl, n-propyl, ethyl, methyl.[94] It was suggested that injury to the erythrocyte cell membrane could result from interaction between the mercurials and the lipophilic components of the membrane. Benesch and Benesch[93] found that larger amounts of mercurials (Salyrgan and phenyl mercury) were required to cause hemolysis than to form a monolayer on the membrane surface. They suggested that hemolysis resulted from structural changes caused by reaction between membrane sulfhydryl groups and mercurials.

Lindahl and Hell[84] reported tissue injuries in gills of fish exposed 10-40 minutes in 0.3×10^{-5} M phenyl mercury. After 10 minutes exposure the outer epithelial layer separated from the gill tissue because of dissolution of the basement layer. By 40 minutes a decrease in circulation in the secondary gills was observed. After 49 minutes, only the phalanges of the pillar of secondary filaments remained. Mucous was found on the gill filaments. They found that the gill filaments were highly penetrable to phenyl mercury. Rucker and Amend[85] observed hyperplasia of the gills of trout and salmon exposed to ethyl mercury.

Lindahl and Hell[84] measured respiration in secondary gill filaments to assess effects of mercurials on gill function. They found that oxygen consumption of the gills was reduced by 30% whether the exposure period was 40 or 60 minutes. The secondary gill filaments composed about 35% of the total gill filaments. They suggested that the functional and structural changes of the gill were the ultimate cause of fish death since it prevented the fish from obtaining adequate oxygen.

EYE LENS

Kipling[19] suggested that local absorption of mercury could cause mercurialentis. The condition has two phases. The first involves a greyish granular discoloration of the anterior capsule of the lens. In the second, the anterior lens blooms and, when observed through a slit lamp, appears brownish-grey, deep rose-brown, pinkish copper, or golden. These characteristics occur at low levels where other evidence of mercury exposure does not. In addition, a band-shaped opacity of the cornea may occur. Individuals showing this type of lesion usually already have mercurialentis. Stal lines have also been attributed to mercury.

SUMMARY

The neurological and renal disturbances observed in living organisms have been shown to result from extensive brain and renal damage. Other tissue effects have been observed which do not contribute as

greatly to the development of gross symptoms. Among these are heart muscle damage and changes in corneal appearance. In addition, studies of blood uptake have given insight into the transport and subsequent distribution of different mercurials.

SECTION V

CELLULAR EFFECTS

Many investigators have attempted to understand the effects of mercurials on tissues by determining the extent of cell damage and the interaction between mercurials and cellular components. Cellular distribution of mercurials, their effects on cell morphology and cellular components (i.e., chromosomes, membranes, mitochondira) are topics researchers have considered.

INTRACELLULAR DISTRIBUTION

Intracellular distribution has been studied by several investigators. Kanzantis[17] suggested that the lysosome may be one of the main organelles which concentrate mercurials. Norseth[95] found 26.6% of the cell mercury in the mitochondrial fraction, 36% in the lysosomes and perioxisomes, and 37.4% in the microsomes after the cells were exposed to methoxyethyl mercury. Using a multi-term equation which included data from marker enzymes, he found 11.7% of the cell Hg in the microsomes, 3.5% in the nuclear material and 1.6% in the supernatant. In a similar report,[10] he compared mercuric chloride, methyl mercury, and methoxyethyl mercury. After mercuric chloride exposure, lysosomes showed the highest concentration. The mitochondria were second, and the microsomes contained the least. The microsomal fraction was greater with methyl mercury. Distribution was more even with methoxyethyl mercury. The microsomal and lysosome-peroxisome fractions contained approximately equal percentages. The mitochondrial percentages were lower.

Comparisons of the distribution of mercurials with increased time after exposure have been made. Norseth[10] reported distribution *vs.* time for mercuric chloride. At one hour after exposure of the liver, the mitochondria and microsomes contained equal percentages of mercury. The lysosomes contained less than half that amount. After one day, all three categories contained approximately equal percentages. By four days, the lysosomes and peroxisomes contained the highest percentage of the liver mercury content. The mitochondria contained the second highest and the microsomes least, with about half the percentage of the lysosomes. Yoshino[81a] studied the intracellular distribution of methyl mercury with increasing time after exposure of brain cells. At six hours, the highest concentration was found in the mitochondria and the second highest in the microsomes. The supernatant contained the least mercury. After day one, the mitochondria contained a slightly higher percentage than the other two and the microsomal and supernatant contained equal percentages. When mercury/mg N was calculated, Yoshino[81a] found the same pattern of distribution at six hours. At day one, no difference in mercury/mg N was observed.

Ellis and Fang[96] compared distributions in liver cells versus kidney cells, phenyl mercury versus mercuric ions, dosage effect, and effect of time after incubation. They observed a difference in nuclear and soluble fractions between kidney and liver cells. No differences in the mitochondrial and microsomal fractions between kidney and liver cells was observed. Changes in distribution were not observed at increasing dosage levels for either. In contrast, Rao et al.[97] observed a change in distribution with substrate concentration. When dosage was increased, the nuclear and microsomal fractions showed little change; however, the mitochondria showed a greater percentage uptake and the soluble fraction, a lower percentage uptake. Accumulation was dosage dependent.[96, 97] No difference in distribution with increasing time after exposure were observed. Comparison of the two mercurials revealed that greater uptake of phenyl mercury than mercuric ions occurred in all fractions after the first six hours. Phenyl mercury was accumulated and lost more rapidly. Ellis and Fang[96] suggested that the greater toxicity of phenyl mercury was due to its greater uptake and storage.

Other variables such as mercuric concentration, incubation time, and temperature could influence mercurial distribution. Rao et al.[97] studied these factors for phenyl mercury and a mercuric salt. Mercury uptake increased with time of incubation for both mercurials; however, longer exposure time resulted in a greater uptake by mitochondria of phenyl mercury than of the mercuric salt. They also suggested, as did Ellis and Fang,[96] that this difference in uptake could be responsible for the difference in toxicity of these compounds. Initially a rapid phase of uptake was observed followed by a slower phase. A non-linear increase in mercury accumulation with increasing temperature from 3-37°C was observed for both mercurials. At 37°C a reduction in uptake was observed for phenyl mercury but not for mercuric chloride. Ellis and Fang[96] suggested that the phenyl mercury might damage the absorption mechanism.

CELL MORPHOLOGY

Miyakawa and Deshimaru[98] followed the microscopic pathological changes in brain tissue after mercurial exposure. They observed changes in occurrence of intracellular organelles. At 12-13 days, though no pathological changes were observed in brain tissues, lysosomes were more numerous in the granule cells bordering the deep sulcus of the cerebellar vermis. By 19-20 days this granule layer showed marked localized changes. At this time the Purkinje and Golgi cells showed an increase in lysosomes. The more prominent changes observed in the granule cells included shrunken nuclei and peripheral vacuolation. Kim[83] observed similar changes in a myelinating culture. A progression of more minute changes were observed using microscopy. The ribosomes disappeared and the intranuclear substance increased. Observations revealed intracellular pathological changes of two

types. In the first, the nuclear membrane gradually concentrated with disappearance of the cell organelles such as the golgi apparatus and the ribosomes. In the second, the nuclear membrane degenerated and the intranuclear substance streamed out. The first was more frequently observed. In both cases, the mitochondria appeared to be the most resistant organelles.

CELL MEMBRANE

Rothstein[99] stated that the cell membrane is the most probable site of damage rather than the enzymes within the cell. He described two aspects of the action of heavy metals on the membranes--chemical reaction with groups on the membrane and resulting physiological disturbances. During the rapid phase of mercury uptake by cells, the mercury binds to the membrane. The reaction is reversible and can inhibit phenomena associated with the surface enzymes, permeability barrier, alteration of bioelectric potential, and changes in surface transport systems. A slower, only partially reversible phase of mercury uptake, indicates incorporation of mercury within the cell. Evidence of the effect on the first phase of uptake can be obtained by looking at inhibition of glucose transport. Evidence of second inhibition can be gained from respiration studies. Demis and Rothstein[100] described the application of this approach to the inhibition of rat diaphragm. The extreme action of mercury on the membrane is irreversible breakdown. The increase in permeability resulting from membrane breakdown is an all or none phenomenon for individual cells.[101] When mercurials bind to the membrane in large enough quantity, a threshold stress level is reached and the cell membrane breaks down.[99,101]

CHROMOSOMES

Reported effects of mercurials on chromosomes are chromosomal breakage, radiomimetic effects, and c-mitosis. Skerfving et al.[102] examined chromosomes of humans exposed to methyl mercury by eating fish. They found a significant rank correlation between the frequency of cells with chromosome breaks and mercury concentration. They were unable to show a significant increase in polyploidy and aneuploidy in humans. Fiskesjo[103] stated that alkyl mercurials were not good inducers of polyploidy because of their great toxicity. Ramel[11] also found chromosome bridges and fragments in Allium after exposure to 0.25×10^{-6} M phenyl and methyl mercury.

Other authors have used c-mitosis as an index of chromosome damage. Ramel[11] described c-mitosis as strong contraction of the chromosome with delayed division of the centromere which gives rise to a cross-like chromosome configuration. They have described the appearance of cells after different concentrations of mercurials and the threshold of c-mitosis. Ramel[11,104] generally described the

cytological effect of different mercurials. At high concentration, fixation of cells results. At lower, but still high concentrations, extensive cell death occurs. C-mitosis results from exposure to still lower concentrations. Spindle abnormalities such as multipolar mitosis may result from concentrations too low to cause c-mitosis. Methyl mercury and methoxyethyl mercury caused similar effects.[105] Fiskesjo[105] described the effects of different concentrations. At 200-2000 x 10^{-6} M all mitosis showed lethal effects. Some showed signs of heterochromaty. At 10-100 x 10^{-6} M, c-mitosis dominated. The control level was reached at 1 x 10^{-6} M. Ahmed and Grant[106] observed c-mitosis in Vicia faba and Trandescantia root tips after exposure to methyl mercury. Polyploid and multinucleate cells were observed but chromosome fragments were absent.

The toxic threshold is characterized by partial inactivation of the spindle.[103] The threshold is least for methyl mercury, ethyl mercury is next, methoxyethyl, and butyl mercury is highest. Ramel[11] reported a threshold of 8 x 10^{-7} M at 72 hours for phenyl mercury. He stated that the threshold for organic mercurials is .005 of that for inorganic mercurials.

Ramel and Magnusson[107] have also reported effects on chromosome segregation. They found that high but non-lethal concentrations gave rise to abnormal wing positions in emerging Drosophila. Higher concentrations cause reduced mobility or failure to emerge. As 25 mg/L methyl mercury, phenyl mercury, and methoxyethyl mercury, exceptional female offspring resulted. Twice as high a dose yielded some exceptional males. He found that methyl and phenyl mercury were similar in levels required to cause exceptional offspring, but five times as much methoxyethyl was required. Female exceptions were usually in the form of xxy exceptions. Male xo exceptions were found. The effect is apparently primary disjunction at the first mitosis.

MITOCHONDRIA

Lehninger[108] suggested that mercury may be an important agent in mitochondrial leakage since it causes swelling in the mitochondrial membrane. The swelling may be induced by changes in the secondary or tertiary structure of proteins in the mitochondrial membrane resulting from the binding of mercuric ions to membrane sulfhydryl groups. The increase in membrane permeability caused by the swelling results in leakage of pyridine nucleotides. Only oxidized NAD is lost.

SECTION VI

BIOCHEMICAL EFFECTS

The action of mercurials at different levels of organization (i.e., the organism, the tissue, the cell) could be explained through an understanding of the effects of mercurials on cellular enzymes and enzyme systems if adequate knowledge were available. The purpose of this section is to clarify enzymatic effects both in terms of possible action of mercurials on enzymes in general and their effects on specific groups of enzymes. Cases in which organ damage can be related to biochemical changes resulting from mercurial exposure will be discussed. More important, however, is the understanding of the problems in generalizing from molecular changes to tissue damage which can be gained from review of biochemical effects.

GENERAL EFFECTS

Webb (Chapt. 4)[109] has discussed reactions between enzymes and mercurials. Chapters 4 and 7 of his book provide the most complete overview of mercurial inhibition available. First, he emphasized the variety of groups of chemical compounds which can react with mercurials. Low molecular weight thiols (i.e., co-factors and amino acids), non-enzyme proteins, and enzymes can all be affected by mercurials. Thus, energy metabolism, cell structural proteins, and a variety of cellular processes could be inhibited by mercurials. However, the reactivity of mercurials with sulfhydryl groups and the cellular consequences of these reactions depend on a number of factors.

Differential activity of sulfhydryl groups and changes in reactivity following denaturization are among the factors important to the reaction between mercurials and sulfhydryl groups. Sulfhydryl groups involved in the tertiary structure of the protein may not be readily available to react with mercurials. Disulfide linkages are less reactive with mercurials than sulfhydryl groups. Different reactivities of free sulfhydryl groups may be influenced by several factors. Steric interference or electrostatic interaction may impede a reaction. The ionization state may influence reactivity with particular sulfhydryl groups. Once the mercurial reacts with a sulfhydryl group other factors become important in determining the effect of the mercurial on the enzyme's functional properties. The mercurial can combine with a sulfhydryl group at an active site on the enzyme. If the mercurial combined with a sulfhydryl group vicinal to the active site, the other group to which the mercurial is bound could sterically or electrostatically interfere with the approach of the enzyme substrate to the active site. The reacted sulfhydryl groups could be involved with maintaining the enzyme

structure. Changes in enzyme structure resulting from the reaction between sulfhydryl groups and mercurials produce a continuum of events ranging from initial reversible reactions between sulfhydryl groups and mercurials to the unfolding of the polypeptide helix which alters the reactivity of the remaining sulfhydryl groups and finally causes precipitation of the protein.

Several physical factors can also influence mercurial reaction with sulfhydryl groups and subsequent inhibition. Hydrogen ion concentration effects the ionization of sulfhydryl groups and thus the competition between the hydrogen and mercuric ions for the sulfur. Several different species of mercurials derived from the one injected are found. For example, injection of mercuric chloride yields mercuric ions, mercuric chloride, mercuric hydroxychloride, and mercuric hydroxide. The pH alters hydroxyl ion concentration and thus influences the quantities of each chemical species found. Protein charge is influenced by pH; therefore, changes in attraction or repulsion of different mercurials could occur at different pH values. Protein reaction with mercurials is usually increased as pH is reduced. The pH determines the state of aggregation of protein-mercurial complexes and the rate of secondary denaturation of the protein. Changes in pH may not affect the reactivity of different sulfhydryl groups in the same way.

CHEMICAL PROPERTIES--ORGANIC MERCURIALS VS. INORGANIC MERCURIALS

Studies of organismal, tissue and cellular effects have suggested that organic mercurials act differently from inorganic ones. Webb (Chapt. 7)[109] discussed important differences in chemical properties. Mercuric chloride can react with two ligands while organic mercurials can only react with one. Organic mercurials have a lower water solubility than inorganic ones. Unsubstituted aryl and alkyl mercurials are more lipid soluble than mercuric chloride, thus, enhancing their tissue penetrability. Their bond configurations are different. Mercuric chloride is linear while organic C-Hg-X have a bond angle of 130° or greater. Organic mercurials have a greater molecular size. Therefore, steric factors may impede their reaction with sulfhydryl groups. Affinities for ligands are somewhat less for organic mercurials than for mercuric ions.

INHIBITION BY MERCURIALS

Webb (Chapt. 7)[109] has discussed several categories of mercurial inhibition. The division has been based on the number of sulfhydryl groups reacted before inhibition occurs. He discussed the following types: (1) inhibition runs parallel to sulfhydryl groups reacted, (2) reaction of sulfhydryl groups does not yield inhibition, (3) inhibition occurs only after a set number of sulfhydryl groups have reacted, (4) inhibition is complete before all groups react, (5) inhibition is parallel to reaction of sulfhydryl groups but saturation

of sulfhydryl groups does not yield complete inhibition, and (6) inhibition occurs without the reaction of sulfhydryl groups.

Citing several authors work, Webb[109] summarized several enzymes which fit the first type. Inhibition was proportional to the number of sulfhydryl groups reacted in several dehydrogenases (lactate, malate, 3-phosphoglyceralde, succinate) and pyrophosphatase. Several enzymes which have single groups at their active center appear to be inhibited by type 1 inhibitors. Webb included glycerol phosphate dehydrogenase and ficin in this group. Sanner and Pihl[110] discussed papain. They found a single reactive sulfhydryl group essential to the enzyme's activity and inhibition was of the type 1. Fasella and Hammes[111] concluded that sulfhydryl groups were not actually involved in the activity of hexokinase after titrating the enzyme with a mercurial. Webb[109] therefore placed this in the second category--enzymes in which the reaction of sulfhydryl groups does not yield inhibition. Webb also placed enolase and catalase in this group. He included ATP-ase, alcohol dehydrogenase, aldolase, B-amylase, phosphorylase, rodanase, urease, and xanthin oxidase in group 3 in which inhibition occurs after a specific number of sulfhydryl groups are bound. Gilmour and Gilbert,[112] studying rabbit myosin, found activation up to a concentration of 3 M mercury/10^5 g myosin. Higher concentrations yielded inhibition. DNA is also not affected until a critical level of bound sulfhydryl groups is reached.[113] The fourth type in which inhibition is complete before all groups react is shown by cytochrome c-reductase, and malate dehydrogenase. Rajagopalan et al.[114] studied hepatic aldehyde oxidase and found that two sulfhydryl groups per mole reacted rapidly with p-mercuribenzoate resulting in inactivation of the enzyme. Therefore, Webb[109] included aldehyde oxidase of rabbit liver in this category. In addition, Marshall et al.[115] found that carbamyl phosphate synthetase is greatly inhibited before sulfhydryl groups are saturated. Several enzymes show inhibition proportional to the number of sulfhydryl groups reacted but are not totally inhibited when all react. Webb[109] has included lactate dehydrogenase from pig muscle, phosphoglucomutase and phosphorylase in the group. The last possible type is not clearly demonstrated by any enzyme.[109]

The different rates at which inhibition occurs are dependent on several factors. Webb (Chapt. 7)[109] discussed these. The more slowly developing inhibition could be caused by less available sulfhydryl groups reacting or secondary denaturation. The differences in time required for inhibition (i.e., 1-2 minutes for succinate dehydrogenase inhibition vs. 5-20 minutes for enolase) are caused by the following factors: (1) relationship between sulfhydryl groups and enzyme activity, (2) different reactivities of different sulfhydryl groups, (3) tendency to undergo structural changes which could lead to inactivation.

Few enzymes are resistant to mercury. Webb (Chapt. 7)[109] reviewed many articles and found several enzymes inhibited less than 10%. Among these were most alkaline phosphatases, many proteases (i.e., trypsin[116]) and peptidases, some pyrophosphatases, most RNA-ases and DNA-ases. He stated that most enzymes or enzyme groups should be discussed more specifically. The basic organization of Webb will be used since he dealt with the more important enzymes about which much information was known.

Many electron transport enzymes are sensitive to mercurials. Van Eys et al.[117] observed 93% inhibition of glycerol phosphate dehydrogenase with 1×10^{-5} M p-chloromercuribenzoate and 100% inhibition with 1×10^{-4} M.[12] Yeast alcohol dehydrogenase was inhibited 50% by 1.5×10^{-7} p-chloromercuribenzoate. Conversely, 10^{-5} M methyl mercury stimulated L-glutamate dehydrogenase[118,119] though it inhibited alanine dehydrogenase.[119] Nishida and Yielding[118] hypothesized that a shift in enzyme conformation caused the inhibition effect. The sensitivity of pre-cytochrome enzymes of the electron transport chain suggests that these are the sites of mercurial action.[109] However, Webb (Chapt. 7)[109] developed his discussion further and showed that the cytochrome system is not immune to mercurial action. Cytochrome oxidase is inhibited 81-98% at 160×10^{-5} M of a variety of mercurials.[120] Lucier et al.[121] and Lucier et al.[122] have investigated changes in a liver cytochrome resulting from methyl mercury exposure. They have found that mercury can stimulate synthesis of cytochrome 450 while increasing degradation yields a decrease in the total amount of cytochrome 450 present. In addition, Webb[109] stated that cytochrome inhibition was not reversed by thiols. Thus, the electron transport system is sensitive to mercurials due to the inhibition of a number of the enzymes of the electron transport chain.

Webb (Chapt. 7)[109] stated that mercurials were not effective uncouplers of oxidative phosphorylation. Shore and Shore[123] observed a reduction in oxidative phosphorylation in rat-kidney mitochondrial system after injection of 3 mg mercuric chloride/kg body weight.

Webb (Chapt. 7)[109] summarized studies of the effects of mercurials on photosynthesis. He stated that the Hill reaction was very sensitive. San Pietro and Lang[124] studied pyridine nucleotide reductase which catalyses the transfer of electrons from the photolytic system to the pyridine nucleotides. They observed 50% inhibition at 1.2×10^{-5} M p-chloromercuribenzoate and 90% inhibition at 1.6×10^{-5} M. Photophosphorylation is not as greatly inhibited. Kahn and Jagendorph[125] studied an enzyme from spinach chloroplasts which probably functions in photophosphorylation. It was inhibited completely at 10^{-3} M mercuric ions. Harriss et al.[126] studying algae showed that small quantities of mercurials result in measurable effects on algal photosynthesis and 50 ppb can cause cessation of photosynthesis in some algae. Lipid biosynthesis, including

chlorophylls, in algae is inhibited by mercurials and may be another factor in reduced photosynthesis.[127]

RATIONALE FOR THE ACTION OF MERCURIALS

Hughes[128] suggested that since the fundamental reaction of mercury is with thiols, the differences in distribution and organ effects is dependent upon this reaction. He discussed the differences in affinity for sulfhydryl groups dependent on chemical species present, large organic functional groups, and charges. These factors serve to direct the mercurial to specific sulfhydryl groups. Peakall and Lincer[129] have suggested a more complex sequence of events. They found reduced numbers of sulfhydryl groups in brain and liver tissues but not in muscle. They suggested that the reduction of sulfhydryl groups was not solely dependent on binding by mercury but also on inhibition of glutathione reductase which interconverts sulfhydryl groups and disulfide linkages. Glutathion reductase activity is negligible in muscle. The work of Pekkanen and Sandholm[130] supported this hypothesis. In addition, in living organisms, distribution is dependent on transport of mercurials and barriers to mercurial uptake by specific organs. Since the blood is rich in thiols, it can transport large amounts of mercurials. Five to ten percent of the thiol content of the plasma is small diffusible compounds. These can diffuse into cells. When in contact with membranes, the differing lipid solubilities among mercurials may determine which dissolve in lipid membranes. Some mercurials can then dissociate from the thiol and pass through the membrane. Hughes[128] suggested this as a rationale for the ready diffusion of methyl mercury. Larger mercurials or bivalently charged ones would not pass so easily through membranes. Thus Hughes suggested that actual pathological effects on human organs is due to the real concentration of mercury in the organs.

Several researchers studying the greater resistance to mercurialism of sucrose-fed rats over chow-fed rats[131, 132, 133] illustrated Hughes' rationale of effect. Surtshin and Yagi[131] found that sucrose- and chow-fed rats three hours after injection had the same mercury content in the kidney. However, the sucrose-fed had a higher content in the soluble fraction and less in the nuclear and granular fractions than the chow-fed. They suggested that the increased resistance could be related to the decreased binding in the renal nuclei and mitochondria. Thus distribution depended on binding to thiols. A greater sulfhydryl group content was found in the soluble fraction of sucrose-fed rats. By binding in the soluble fraction rather than in the granular fractions, some protection was afforded the granular fraction.

IN VIVO EFFECTS--RATIONALE FOR SPECIFIC ORGAN DAMAGE

Webb (Chapt. 7)[109] commented on tissue homogenate effects as opposed to enzyme effects resulting from the action of mercurials on enzymes

of the Krebs cycle. Webb stated that many enzymes of the tricarboxylic acid cycle are sensitive to mercurials. However, work with homogenates, mitochondria, etc., have shown that the non-enzyme proteins provide considerable protection so that the actual effect on the activity of the Krebs cycle is not great. Rothstein[99] emphasized the protection provided by the membrane.

Several researchers have attempted to determine the cause of the severe pathological changes in certain brain cells. Hughes[128] suggested that mercurials do not actually block neuron function but block their metabolism. Hell and Lindahl[134] observed marked inhibition of mitochondrial oxidation of a-ketoglutarate and succinate after *in vitro* exposure to phenyl mercury. However, rats subjected *in vivo* to phenyl mercury showed no differences in mitochondrial oxidation, respiration, or aerobic glycolysis when compared to the control rats. The rats could have still been in the latent period prior to manifesting neurological symptoms, or the absence of effect could have been due to non-enzymatic protein and membrane protection.

Yoshino *et al.*[81b] studied changes in the brain of rats after *in vivo* exposure to methyl mercury. They considered both rats in the latent period and after the manifestation of neurological symptoms. They found marked reduction of parameters measured only in rats showing neurological symptoms. Oxygen consumption was reduced 37%. No difference in anaerobic lactic acid formation was observed in rats with or without neurological symptoms. A 27% decrease in aerobic lactic acid formation was observed in rats manifesting neurological symptoms. In addition, they measured the activities of several sulfhydryl-dependent enzymes in five major areas of the brain. In control rats, succinate dehydrogenase and ATP-ase showed significant differences in activity among the five areas tested while aldolase did not. The activities of succinate dehydrogenase and ATP-ase were lower in the white matter than in other areas. No change in enzyme activities was observed during the latent period. Succinate dehydrogenase was significantly reduced in rats showing neurological symptoms though no difference in the rate of decrease was observed among the five areas. Only a slight decrease (not statistically significant) was observed in ATP-ase activity of rats showing neurological symptoms *vs.* those not. No change in aldolase activity was observed. One process, protein synthesis, was inhibited during the latent period. Leucine incorporation was reduced 57% during the latent period and 42% after neurological symptoms were apparent. Yoshino *et al.*[81b] suggested that the disturbance of respiration would be delayed until the supply of protein became insufficient for the maintenance of cell life. This would require several days since the average half-life of protein is 14 days. Though damage to succinate dehydrogenase and Krebs' cycle may be important in reduced oxygen consumption of the brain, their study indicated that the main biochemical involvement is with reactions essential for protein synthesis. In contrast, Webb[109] stated that protein synthesis was

not especially sensitive to mercurials. Brubaker et al.[135] found enhanced incorporation of amino acids in kidney exposed to methyl mercury and suggested induction of protein synthesis by methyl mercury.

Paterson and Usher[136] studied the effect of methyl mercury on glycolytic intermediates of the rat brain. They studied glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, fructose-1-6-diphosphate, phosphoenolpyruvate, pyruvate, dihydroxyacetone phosphate and the adenine nucleotides (ATP, ADP, and AMP). Dosage levels of 0.05, 0.5, and 5 mg/g were used. The glycolytic intermediates were statistically different from the controls at the two higher dosage levels. Two distinct patterns were apparent. At the highest level, a 13-17% increase in glucose-1-phosphate, dihydroxyacetone phosphate, glyceraldehyde-3-phosphate, and 2- and 3-phosphoglycerate was observed. An 18% increase in a-glycerophosphate was observed at the two higher dosage levels. Other differences were less significant when compared to controls than the highest group. At 0.05 mg/g glucose-1-phosphate was reduced and 3-phosphoglycerate was increased. The a-glycerophosphate was the same as the control. The ratio of ATP to ADP and AMP was lowered with the middle and high dose when compared to their control but raised with the low dose. These differences were not statistically significant. Paterson and Usher[136] stated that the accumulation of glyceraldehyde-3-phosphate, 3-phosphoglycerate, and 2-phosphoglycerate indicated inhibition of enzymes toward the end of the glycolytic path. They suggested that phosphoglycerate mutase, enolase, pyruvate kinase, and pyruvate dehydrogenase were probably the affected enzymes. Thus a decrease in ATP was observed. They discussed the possible alternate path if the glycolytic metabolites were transferred to the mitochondrion for oxidation--the alternate path was the dihydroxyacetone/a-glycerophosphate shuttle. The increase in a-glycerophosphate levels indicated that the alternate path was operating on the glycolytic intermediates; however, since a-glycerophosphate was the same for the two higher mercurial levels, the shuttle was probably operating maximally. They concluded that mercurials have an acute effect on glycolytic intermediates.

The biochemical basis for kidney damage appears to be inhibition of energy-transforming cycles. Shore and Shore[123] measured uptake of whole kidney homogenates. At four hours after exposure, only 20-30% inhibition was observed. By twelve hours after injection of the rats, some showed complete inhibition of oxygen consumption while others retained partial activity. Complete inhibition of the tricarboxylic acid cycle was observed by 24 hours. Oxidative phosphorylation was reduced by 90% by six hours after injection. The a-ketoglutarate dehydrogenase system was inhibited more than succinate dehydrogenase or cytochrome oxidase. Phosphorylation was inhibited more than oxidation. Shore and Shore[123] suggested that

disruption of phosphorylation could be more important to the cycle's breakdown than direct inhibition of Krebs' cycle enzymes. They stated that it was difficult to determine the cause of renal failure, but that without energy supplied by the tricarboxylic acid cycle, the kidney could not function. They also indicated that mercury may be transferred from site to site several hours after maximal kidney concentration. Maximal kidney concentration occurs at three hours and maximal inhibition at 24 hours.

Mercurial diuretics apparently act through the suppression of energy providing enzyme systems thus depressing tubular resorptive mechanisms relying on active transport.[137, 138] Goodman and Gelman[137] suggested that resorption of chloride ions is specifically blocked while Hirsch[138] thought it is a general depression of tubular function.

Hell and Lindahl[134] studied the effects of phenyl mercury on the energy metabolism of liver slices. *In vitro* reduction of mitochondrial oxidation of a-ketoglutarate and succinate, oxygen consumption, and the P/O ratio were observed. No effects were observed in liver slices of rats exposed *in vivo*. *In vitro vs. in vivo* inhibition of several liver enzymes has been compared.[12] Fifty percent inhibition of alkaline phosphotase resulted from exposure to 10^{-5} M mercuric ion. *In vivo* exposure to 0.23 mg/L (the median tolerance limit for 96 hours) resulted in a slight but significant increase in activity. Acid phosphatase showed 40% inhibition at 10^{-4} M. A slight, but a significant decrease was observed *in vivo*.

SECTION VII

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